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THE meteorological service of India is beginning aerological work with balloons sondes.

AN extreme minimum temperature of -91.9° C. was recorded with a balloon sonde on November 5, 1913, over Batavia, Java. Another balloon sonde brought down a record of -90.9° at 17 km. altitude on December 4. Above this the temperature rose to -57.1° at 26 km.⁶

PYRHELIOMETRIC observations obtained from balloons sondes in California last summer at altitudes of 10 to 13 km. indicate a lower solar constant of radiation than is obtained from observations at the earth's surface after transmission corrections have been added. Although a maximum altitude of 33 km. was reached, no observations were secured above 13 km. because of the freezing of the mercury.⁷

THE unpublished papers of the International Meteorological Congress held at Chicago in 1893 are now appearing in the *Monthly Weather Review*.

A CONFERENCE of observers and students of meteorology and allied subjects will be held in Edinburgh, September 8 to 12, 1914.⁸

CHARLES F. BROOKS

HARVARD UNIVERSITY,
May 18, 1914

SPECIAL ARTICLES

A CULTURE MEDIUM FOR THE TISSUES OF AMPHIBIANS

IN the course of some experiments on the culture *in vitro* of tissues from various amphibians, considerable difficulty was encountered in using blood plasma as a culture medium on account of its very rapid coagulation. When working with the tissues of the frog or of tadpoles it was more convenient to use lymph taken directly from some of the subcutaneous lymph spaces. Preparations in lymph may frequently be made before coagulation occurs, but the lymph tends to become too watery for

⁶ *Nature*, March 5, 1914, pp. 5-6.

⁷ C. G. Abbot, *Scientific American*, April 4, 1914, p. 278.

⁸ See *Nature*, February 12, 1914, p. 667.

use a short time after the frog is killed, so that only a small quantity is available from any one animal. In most urodeles the scarcity of available lymph prohibits its employment, so that plasma was at first depended on almost entirely for a culture medium.

There is little outwandering or outgrowth from the tissues of either the embryos or the adults of amphibians unless the surrounding medium is of more or less solid consistency. Amphibian tissue will live for weeks in blood serum or even in Ringer's solution, but the cells do not often grow or wander away from the rest of the mass unless they come into contact with a substance that evokes a thigmotactic response. In searching for a convenient substitute for blood plasma the endeavor was therefore made to find a medium which would remain fluid while being used, but which would solidify to about the consistency of blood clot afterwards. After some experimentation it was found that a mixture of equal parts of blood serum and a two per cent. solution of Grübler's nutrient gelatine afforded a substitute that was very successful.

The preparation of the mixture is easy. Blood drawn from the heart is stirred with a fine glass rod and the coagulum removed. The blood is then centrifuged to remove the corpuscles, and the clear serum mixed with an equal quantity of a two per cent. solution of gelatine. The gelatine solution is previously boiled and precautions are taken to prevent contamination of any of the ingredients of the medium with bacteria. I have used the mixture after it had been kept for several days, and found it to be practically as good a culture medium as when perfectly fresh.

The mixture becomes fluid when warmed slightly and remains fluid for an hour or more after being cooled to ordinary room temperature. I commonly keep it in small tubes of glass, and by rubbing the tubes briskly with the fingers sufficient heat may be generated to cause the gelatine to liquify. Should the supply of culture medium solidify while one is putting up preparations, it is only necessary to warm it slightly to keep it fluid for an hour or more longer.

Making preparations of tissues is greatly facilitated by the use of this medium, and the comparatively constant composition of the mixture renders the results obtained through its use more uniform than those secured by the employment of lymph or plasma. The implanted cells get what very nearly corresponds to their natural food in the serum of the blood, and the gelatine, while apparently acting in no way injuriously to the cells, affords a means of appealing to their thigmotactic propensities that is ordinarily supplied by the fibrin of clotted plasma.

The outgrowth of epithelium in this medium is remarkable. In some cases it has been over twenty times the superficial area of the implanted tissue. As a rule the tissues thrive better than in plasma or lymph. It is comparatively easy to subculture the tissues, since the gelatine dissolves in Ringer's solution, and by washing the preparations in this fluid they may be readily freed, and then transferred to a fresh culture medium. I have transferred pieces of epithelial tissue several times in succession, and kept them thriving for three months. Cell divisions have been repeatedly seen in epithelial cells in this medium. In a piece of tissue put up on February 17 and changed to fresh culture fluid three times afterwards, I observed several mitotic figures in epithelial cells on April 8, fifty days after the preparation was made. The chromosomes could be seen with great distinctness in the living material. In one cell first seen in the prophases of division, the chromosomes were seen to align themselves in the equatorial plate, then to be drawn apart, and finally to become constituted into the two daughter nuclei; at the same time the constriction in two of the cell body could be distinctly followed. Over a dozen other mitotic figures in various stages were observed in the same piece. The preparation had been washed in Ringer's solution and transferred to new culture medium a few days previously, after which it had taken on a new lease of life. The division figures were all seen in a transparent sheet of epithelium that had spread out in contact with

the cover slip supporting the hanging drop culture.

S. J. HOLMES

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIF.

ON THE CHEMICAL NATURE OF THE LUMINOUS
MATERIAL OF THE FIREFLY

OUR knowledge of the chemistry of light production by organisms may be summed up in the statement that phosphorescence is due to the oxidation of some substance formed in the cells of the animal. As with other oxidations, both water and oxygen must be present. If either water or oxygen are absent the photogenic substance will not be used up by oxidation. Luminous tissues if dried rapidly may be ground up and preserved indefinitely, and at any later time, if moistened in the presence of oxygen, will phosphoresce. This old and important discovery makes the investigation of the chemical nature of the luminous substance relatively easy. The dried powder of the luminous organ may be extracted with: (1) Oxygen-free watery solvents, or (2) water-free solvents (as ether, chloroform, etc.) with or without oxygen.

The earlier workers supposed the photogenic material to be phosphorus or phosphine. These views require no comment to-day. Later suggestions have been that the substance is a fat, an albumin, a lipoid (lecithin), a nucleoalbumin or a lecithoprotein (phosphatid). It is obviously desirable to know whether the substance is fat-like or protein in nature. The fact that phosphorescence ceases as soon as the moist luminous material is heated to 100° proves nothing, for, like organic oxidation in general, an oxidizing ferment is probably involved, and it is this oxidase which may be destroyed on heating.

I can state definitely that the "luciferin" of the common fire-fly is not a true fat or any fat-like body such as lecithin. The dried material may be extracted with anhydrous ether and the ether extract evaporated to dryness. On adding water or a watery extract of luminous organ (to add an oxidizing enzyme) or potato juice (to add an oxidase) to the residue no phosphorescence took place; on adding water to the original ether extracted